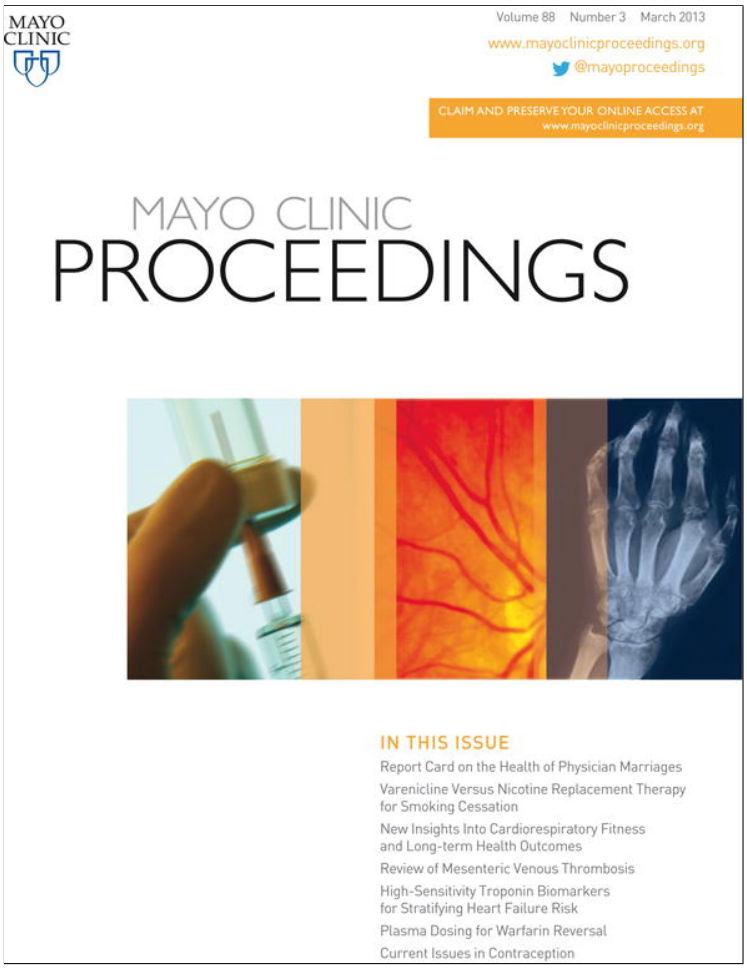

ORIGINAL ARTICLE

MAYO CLINIC

Combined Use of the Novel Biomarkers High-Sensitivity Troponin T and ST2 for Heart Failure Risk Stratification vs Conventional Assessment

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Abstract

Objective: To assess an innovative multimarker strategy for risk stratification of death in a real-life ambulatory heart failure (HF) cohort.

Patients and Methods: The study included 876 consecutive outpatients (median age, 70.3 years; left ventricular ejection fraction, 34%) between May 22, 2006, and July 7, 2010, prospectively followed up in a structured HF unit. A combination of biomarkers reflecting myocardial stretch (N-terminal pro-B-type natriuretic peptide [NT-proBNP]), myocyte injury (high-sensitivity cardiac troponin T [hs-cTnT]), and ventricular fibrosis and remodeling (high-sensitivity ST2 [hs-ST2]) were added to an assessment based on established mortality risk factors (age, sex, left ventricular ejection fraction, New York Heart Association functional class, diabetes mellitus, estimated glomerular filtration rate, ischemic etiology, sodium level, hemoglobin level, and pharmacologic treatment).

Results: During median follow-up of 41.4 months, 311 patients died. The combined addition of hs-cTnT and hs-ST2 to the model yielded good measurements of performance (C statistic, 0.789; Bayesian information criterion, 3611; integrated discrimination improvement, 4.1 [95% CI, 2.5-5.6]; and net reclassification index, 13.9% [95% CI, 6.2-21.6]). Reclassification did not significantly benefit after NT-proBNP addition into the full model; some indices even worsened with all 3 biomarkers. Separate addition of NT-proBNP provided prognostic discrimination only in the subgroup of patients with either hs-cTnT or hs-ST2 levels below the cutoff points (hazard ratio, 2.97; 95% CI, 2.24-9.39; $P<.001$).

Conclusion: A multimarker strategy seems useful for stratifying risk in chronic HF. However, NT-proBNP in addition to the new-generation biomarkers hs-cTnT and hs-ST2 had a limited effect on risk stratification.

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Chronic heart failure (HF) is a growing public epidemic with increasing incidence and prevalence.[1] Despite important progress in recent decades, mortality remains high for patients with HF. Moreover, established risk factors, such as New York Heart Association (NYHA) functional class, medication use, laboratory values, and left ventricular ejection fraction (LVEF) do not fully explain the mortality risk of patients with HF and do not estimate an individual's prognosis.[2-4] Risk stratification may be refined by the use of biomarkers of different pathophysiologic processes that established mortality risk factors do not necessarily directly reflect. However, despite a variety of recently identified novel biomarkers,[5,6] only natriuretic peptides have entered routine clinical practice and been included in clinical guidelines.[1,7] Natriuretic peptides are useful in a wide range of situations,[8] from screening asymptomatic individuals at risk for HF[9] or assessing patients with dyspnea[10,11] to stratifying prognosis in acute and chronic HF[12-14] and even for therapy guidance and monitoring.[15]

A single biomarker might not reflect all the facets of the HF syndrome, and a multimarker strategy may better characterize the complexity of HF.[5,6,16-18] To date, most multimarker strategies in HF have relied on the addition of one

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or more biomarkers to the well-established natriuretic peptides.[6] Whether biomarkers for other pathophysiologic pathways can take the place of natriuretic peptides remains unknown. Accordingly, in the present study, we investigated the value of combining N-terminal pro-B-type natriuretic peptide (NT-proBNP) (a marker of myocardial stretch), high-sensitivity cardiac troponin T (hs-cTnT) (a marker of myocyte injury), and high-sensitivity soluble ST2 (hs-ST2) (reflective of myocardial fibrosis and remodeling) in a large real-life cohort of ambulatory patients with HF. We examined different biomarker combinations in addition to performing an assessment based on established mortality risk factors to determine the relative role of each biomarker in risk stratification.

PATIENTS AND METHODS

Study Population

Between May 22, 2006, and July 7, 2010, ambulatory patients treated at a multidisciplinary HF unit were consecutively included in the study in an outpatient setting. Patients were referred to the unit by cardiology or internal medicine departments and, to a lesser extent, by the emergency or other hospital departments. The principal referral criterion was HF according to the European Society of Cardiology guidelines irrespective of etiology, at least one HF hospitalization, or a reduced LVEF.

Blood samples were obtained by venipuncture between 9 AM and noon during conventional ambulatory visits, and adequate centrifugation serum samples were stored at −80°C. The NT-proBNP, hs-cTnT, and hs-ST2 levels were analyzed from the same blood sample.

All the participants provided written informed consent, and the local ethics committee (Clinical Investigation Ethics Committee, Hospital Universitari Germans Trias i Pujol, Badalona, Spain) approved the study. All the study procedures were in accord with the ethical standards outlined in the 1975 Declaration of Helsinki, as revised in 1983.

Follow-up and Outcomes

All the patients were followed up at regular predefined intervals, with additional visits as required in cases of decompensation, need for up-titration, or other circumstances (such as renal function impairment and anemia) that required closer follow-up. The regular visitation schedule included a minimum of quarterly visits with nurses, biannual visits with physicians, and elective visits with geriatricians, psychiatrists, and rehabilitation physicians.[19,20] Patients who did not attend the regular visits were contacted by telephone. Death from all causes was the main outcome. Fatal events were identified from clinical records or by reviewing the electronic clinical history at the Catalan Institute of Health.

hs-cTnT Assay

Troponin levels were measured by electrochemiluminescence immunoassay using an hs-cTnT assay and the Modular Analytics E 170 system (Roche Diagnostics). The hs-cTnT assay had an analytic range of 3 to 10,000 ng/L. At the 99th percentile value of 13 ng/L, the coefficient of variation was 9%. The analytic performance of this assay has been validated and complies with the recommendations of the ESC-ACCF-AHA-WHF Global Task Force for use in the diagnosis of myocardial necrosis.[21] The assays were run with reagents from lot 157123, which was unaffected by the analytical issues that emerged with Roche hs-cTnT assays.

hs-ST2 Assay

The level of ST2 was measured from plasma samples using a high-sensitivity sandwich monoclonal immunoassay (Presage ST2 assay; Critical Diagnostics). The antibodies used in the Presage assay were generated from recombinant protein based on the human complementary DNA clone for the complete soluble ST2 sequence.[22] The hs-ST2 assay had a within-run coefficient of less than 2.5% and a total coefficient of variation of 4%.

NT-proBNP Assay

The NT-proBNP levels were determined using an immunoelectrochemiluminescence assay and the Modular Analytics E 170 system. This assay has less than 0.001% cross-reactivity with bioactive BNP, and in the constituent studies in this report, the assay had inter-run coefficients of variation ranging from 0.9% to 5.5%.[12]

Statistical Analyses

Categorical variables are expressed as percentages. Continuous variables are expressed

as the mean ± SD or median (25th-75th percentiles [P_{25-75}]) according to normal or nonnormal distribution. Statistical differences between groups were compared using the χ^2 test for categorical variables.

The best cutoff points for hs-cTnT, hs-ST2, and NT-proBNP were found by bootstrapping the value that maximized the log-likelihood of the nonadjusted Cox models. Log-rank tests for Kaplan-Meier survival curves were performed for testing differences among the best NT-proBNP, hs-cTnT, and hs-ST2 cutoff points.

Survival analyses were performed using Cox regression models. To fulfill the assumption of linearity of the covariables hs-cTnT, hs-ST2, and NT-proBNP, the logarithmic functions of both NT-proBNP and hs-cTnT, the quadratic term of the logarithmic function of hs-cTnT and the quadratic term of hs-ST2 were used in the Cox models. The following variables were incorporated into the model: age, sex, LVEF, estimated glomerular filtration rate (eGFR), body mass index, NYHA functional class, diabetes mellitus, chronic obstructive lung disease, atrial fibrillation, ischemic etiology, plasma hemoglobin level, serum sodium level, β-blocker treatment, and angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) treatment. These variables were chosen because they were significant in the univariate analysis; if nonsignificant, they were considered to be of clinical importance (such as sex, LVEF, and ischemic etiology).

We used different measurements of performance to test the potential incremental prognostic value of these biomarkers, as follows.

Discrimination. The area under the receiver operating characteristic curve (AUC) summarized the diagnostic discrimination. Discrimination refers to a model's ability to correctly distinguish 2 classes of outcomes. We used the index of rank correlation, Somers D, which already incorporates information of censored data. The AUCs between models were compared using the *U* test for equality concordance.

Calibration. (1) The D'Agostino-Nam version of the Hosmer-Lemeshow calibration test was used to calculate a χ^2 value. A model is well calibrated when predicted and observed values agree for any reasonable grouping of the observation (no statistically significant differences in the Hosmer-Lemeshow test results). (2) The Bayesian information criterion (BIC), the Akaike information criterion (AIC), and the Brier score were calculated for each model. The AIC and BIC are measures of the relative goodness of fit of a statistical model. The BIC penalizes free parameters more strongly than does the AIC. The Brier score measures the average squared deviation between predicted probabilities for a set of events and their outcomes, so a lower score represents higher accuracy. It takes values between 0 and 1. Given any 2 estimated models, the model with the lower BIC, AIC, and Brier scores was preferred. No statistical tests compare different BIC, AIC, or Brier score estimations, and lower values indicate a better model. (3) The global goodness of fit of the models was evaluated by likelihood ratio tests. A significant *P* value in this test means that adding a new variable to the model significantly improves the accuracy of the model.

Reclassification. We used the method described by Pencina et al.[23] There are 2 main statistics to assess reclassification. Integrated discrimination improvement (IDI) considers the changes in the estimated mortality prediction probabilities as a continuous variable. Two-sided $P<.05$ was considered statistically significant. Net reclassification improvement (NRI) requires a previous definition of meaningful risk categories (we used tertiles for the risk of death: <18.5%, 18.5%-41%, and >41%). The NRI considers changes in the estimated mortality prediction probabilities that imply a change from one category to another.

All the analyses were performed using the software R (version 2.11.1) statistical package (Foundation for Statistical Computing).

RESULTS

Among the 891 consecutive patients included between May 22, 2006, and July 7, 2010, the 3 biomarkers (hs-cTnT, hs-ST2, and NT-proBNP) were available in 876, who were finally included in this analysis. The median patient age was 70.3 years (P_{25-75}, 60.5-77.2 years). Table 1 shows the baseline characteristics of the entire sample. During median follow-up of 41.4 months (P_{25-75}, 22.1-60.5 months), 311 patients died. Of the cardiovascular causes of death (168), refractory HF was responsible in 91 patients (54.1%), sudden death in 30 (17.8%), and acute

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TABLE 1. Baseline Demographic and Clinical Characteristics of Patients With HF and Treatments During Follow-up[a,b]

Characteristic	Total (N=876)	Alive (n=565)	Deceased (n=311)	HR_{Cox} (95% CI)	*P* value
Age (y)[c]	70.3 (60.5-77.2)	66.1 (56.5-74.3)	75.6 (69.9-81.0)	1.07 (1.06-1.08)	<.001
Female, No. (%)	246 (28.1)	151 (26.7)	95 (30.5)	1.07 (0.84-1.36)	.58
White race, No. (%)	871 (99.4)	560 (99.1)	311 (100)	20 (0.04-11,902)	.34
Etiology, No. (%)					.005
Ischemic heart disease	456 (52.1)	288 (51.0)	168 (54.0)	1	
Dilated cardiomyopathy	86 (9.8)	67 (11.9)	19 (6.1)	0.57 (0.36-0.92)	.02
Hypertensive	83 (9.5)	44 (7.8)	39 (12.5)	1.29 (0.91-1.83)	.15
Alcoholic cardiomyopathy	50 (5.7)	40 (7.1)	10 (3.2)	0.53 (0.28-1.01)	.05
Toxic	22 (2.5)	15 (2.7)	7 (2.3)	0.88 (0.41-1.88)	.74
Valvular	102 (11.6)	56 (9.9)	46 (14.8)	1.37 (0.99-1.90)	.06
Other	77 (8.8)	55 (9.7)	22 (7.1)	0.79 (0.51-1.24)	.31
HF duration (mo)[c]	27.3 (4.9-73.8)	24.9 (3.6-67.7)	36 (9-88.1)	1.00 (1.00-1.00)	.02
HF hospitalizations in the previous mo, No. (%)	522 (59.6)	341 (60.4)	181 (58.2)	0.96 (0.77-1.20)	.71
LVEF (%)[c]	34 (26-43)	35 (26-43)	34 (25-45)	1.00 (0.99-1.01)	.79
LVEF ≥45%, No. (%)	202 (23.1)	124 (21.9)	78 (25.1)	0.98 (0.76-1.27)	.89
eGFR (mL/min/1.73 m^2)[c]	42.4 (29.3-59.5)	49.6 (34.7-66.1)	33.5 (23.4-44.5)	0.97 (0.96-0.97)	<.001
BMI[c]	26.9 (24.2-30.5)	27.1 (24.7-30.7)	26.4 (23.6-29.8)	0.95 (0.93-0.98)	<.001
NYHA functional class, No. (%)					<.001
I	63 (7.2)	59 (10.4)	4 (1.3)	1	
II	574 (65.5)	414 (73.3)	160 (51.4)	5.60 (2.08-15.11)	<.001
III	230 (26.3)	90 (15.9)	140 (45.0)	16.19 (5.98-43.77)	<.001
IV	9 (1.0)	2 (0.4)	7 (2.3)	25.30 (7.39-86.66)	<.001
Hypertension, No. (%)	536 (61.2)	330 (58.4)	206 (66.2)	1.35 (1.06-1.70)	.01
Diabetes mellitus, No. (%)	314 (35.8)	182 (32.2)	132 (42.4)	1.44 (1.15-1.81)	.001
COLD, No. (%)	148 (16.9)	73 (12.9)	75 (24.1)	1.67 (1.29-2.16)	<.001
SAHS, No. (%)	39 (4.5)	28 (5.0)	11 (3.5)	0.80 (0.44-1.46)	.46
Atrial fibrillation, No. (%)	146 (16.7)	83 (14.7)	63 (20.3)	1.45 (1.10-1.91)	.009
Treatments (follow-up), No. (%)					
ACEI or ARB	785 (89.6)	529 (93.6)	256 (82.3)	0.34 (0.26-0.46)	<.001
β-Blocker	767 (87.6)	528 (93.5)	239 (76.8)	0.36 (0.27-0.47)	<.001
Spironolactone/eplerenone	344 (39.3)	226 (40.0)	118 (37.9)	1.05 (0.84-1.32)	.66
Loop diuretic	743 (84.8)	458 (81.1)	285 (91.6)	2.25 (1.51-3.68)	<.001
Digoxin	269 (30.7)	158 (28.0)	111 (35.7)	1.33 (1.06-1.68)	.02
CRT, No. (%)	47 (5.4)	31 (5.5)	16 (5.1)	0.83 (0.50-1.38)	.47
ICD, No. (%)	92 (10.5)	66 (11.7)	26 (8.4)	0.69 (0.46-1.03)	.07
Sodium (mmol/L)[c]	139 (137-142)	140 (137-142)	139 (137-141)	0.93 (0.90-0.96)	<.001
Hemoglobin (g/dL)[d]	12.9±1.8	13.3±1.8	12.3±1.8	0.81 (0.77-0.85)	<.001
NT-proBNP (ng/L)[c,e]	1361 (510-3012)	965 (361-2376)	2215 (935-5193)	1.62 (1.49-1.76)	<.001
hs-cTnT (ng/L)[c,e]	22.6 (10.6-40.6)	15.7 (7.9-30.9)	34.2 (20.7-53.7)	11.68 (5.51-24.75)	<.001
hs-ST2 (ng/L)[c,e]	38.1 (30.8-50.9)	35.4 (29.3-45.5)	44.7 (34.1-61.0)	1.04 (1.03-1.05)	<.001

[a]ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; BMI = body mass index (calculated as weight in kilograms divided by height in meters squared); COLD = chronic obstructive lung disease; CRT = cardiac resynchronization therapy; eGFR = estimated glomerular filtration rate; HF = heart failure; HR_{Cox} = Cox model hazard ratio; hs-cTnT = high-sensitivity circulating troponin T; hs-ST2 = high-sensitivity soluble ST2; ICD = implantable cardioverter-defibrillator; LVEF = left ventricular ejection fraction; NT-proBNP = N-terminal pro-B-type brain natriuretic peptide; NYHA = New York Heart Association; SAHS = sleep apnea–hypopnea syndrome.

[b]SI conversion factor: To convert hemoglobin values to g/L, multiply by 10.0.

[c]Data are expressed as median (25th-75th percentiles).

[d]Data are expressed as mean ± SD.

[e]The logarithmic functions of NT-proBNP and hs-cTnT, the quadratic term of the logarithmic function of hs-cTnT, and the quadratic term of hs-ST2 were used in the Cox models. hs-$ST2^2$: *P*<.001; log(hs-cTnT)^2: *P*<.001.

myocardial infarction in 15 (8.9%). Two patients were lost to follow-up and adequately censored.

Cox Regression and Modeling

In the bivariate analysis, the 3 biomarkers predicted death from all causes as continuous variables: log(NT-proBNP) (hazard ratio [HR], 1.62; 95% CI, 1.49-1.76; $P<.001$); log(hs-cTnT) (HR, 11.68; 95% CI, 5.51-24.75; $P<.001$); and hs-ST2 (HR, 1.04; 95% CI, 1.03-1.05; $P<.001$). In multivariate analysis, the 3 biomarkers remained independent predictors of mortality together with age, NYHA functional class, β-blocker treatment, and hemoglobin level (Table 2). When only cardiovascular death was analyzed, log(hs-cTnT) (HR, 7.07; 95% CI, 2.21-22.65; $P=.001$) and hs-ST2 (HR, 1.02; 95% CI, 1.0-1.04; $P=.04$) remained independently associated with cardiovascular mortality, whereas log(NT-proBNP) did not (HR, 1.15; 95% CI, 0.96-1.37; $P=.13$).

Density plots of the best cutoff points in nonadjusted Cox models were calculated using bootstrap methods to identify optimal prognostic cutoff points for NT-proBNP (1720 ng/L; 95% CI, 1550-2000 ng/L), hs-cTnT (16 ng/L; 95% CI, 14-29 ng/L), and hs-ST2 (50 ng/L; 95% CI, 37-85 ng/L). Two-by-two combinations of biomarkers showed that patients with hs-cTnT + hs-ST2 levels above the cutoff points had the highest risk (HR, 11.69; 95% CI, 7.81-17.49; $P<.001$). Kaplan-Meier survival curves according to hs-cTnT and hs-ST2 levels are shown in Figure 1, A. The separate addition of NT-proBNP provided prognostic discrimination only in patients with either hs-cTnT or hs-ST2 below the cutoff point (HR, 2.97; 95% CI, 2.24-9.39; $P<.001$). In patients whose hs-cTnT + hs-ST2 levels were above the cutoff points, NT-proBNP incorporation had a null effect (HR, 1.43; 95% CI, 0.92-2.29; $P=.11$) (Figure 1, B).

TABLE 2. Multivariate Cox Regression Analysis[a]

Variable	HR	95% CI	*P* value
Age	1.04	1.02-1.05	<.001
Female	0.76	0.58-1.01	.06
Ischemic etiology of HF	1.06	0.83-1.36	.65
LVEF	1.00	0.99-1.01	.60
NYHA functional class	1.69	1.32-2.17	<.001
eGFR	1.00	0.99-1.01	.81
BMI	1.00	0.98-1.03	.87
Diabetes mellitus	1.18	0.93-1.50	.17
COLD	1.10	0.98-1.03	.87
Atrial fibrillation	0.94	0.70-1.27	.68
ACEI or ARB treatment	0.81	0.57-1.15	.24
β-Blocker treatment	0.56	0.41-0.76	<.001
Sodium level	0.97	0.94-1.00	.07
Hemoglobin level	0.92	0.86-0.98	.02
log(NT-proBNP)[b]	1.15	1.01-1.31	.03
hs-ST2[b]	1.02	1.01-1.03	<.001
log(hs-cTnT)[b]	3.90	1.81-8.41	.001

[a]ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; BMI = body mass index; COLD = chronic obstructive lung disease; eGFR = estimated glomerular filtration rate; HF = heart failure; HR = hazard ratio; hs-cTnT = high-sensitivity circulating troponin T; hs-ST2 = high-sensitivity soluble ST2; LVEF = left ventricular ejection fraction; NT-proBNP = N-terminal pro-B-type brain natriuretic peptide; NYHA = New York Heart Association.

[b]The logarithmic functions of NT-proBNP and hs-cTnT, the quadratic term of the logarithmic function of hs-cTnT, and the quadratic term of hs-ST2 were used in the Cox models. hs-ST2^2: $P=.001$; log(hs-cTnT)^2: $P=.004$.

Measurements of Performance

Discrimination. The AUC for the prediction of death increased significantly when any combination of 2 biomarkers, or all 3, was incorporated into the model with established mortality risk factors (age, sex, LVEF, NYHA functional class, diabetes mellitus, eGFR, ischemic etiology, sodium level, hemoglobin level, β-blocker treatment, and ACEI or ARB treatment) (models 2-5 in Table 3). The AUC for hs-cTnT + hs-ST2 was similar to that for NT-proBNP + hs-cTnT + hs-ST2 (model 4 vs model 5 in Table 4).

Calibration. The *P* values for the Hosmer-Lemeshow statistics indicated good calibration for all the models with and without biomarkers ($P>.12$ for all the comparisons) (Table 3). The BIC, AIC, and Brier scores were lower in the models that included hs-cTnT + hs-ST2 and the combination of the 3 biomarkers (Table 3). Global goodness of fit was better in models including biomarkers than in the model with only established mortality risk factors as evaluated by likelihood ratio tests ($P<.001$) (Table 3). The separate addition of NT-proBNP (model 4 vs model 5 in Table 4) improved global goodness of fit in the total population (likelihood ratio, $P=.04$). However, in the subgroup of patients whose hs-cTnT + hs-ST2 levels were equal to or above the cutoff points, the likelihood ratio was not significant ($P=.11$).

Reclassification. The IDI (risk as a continuous variable) increased significantly with

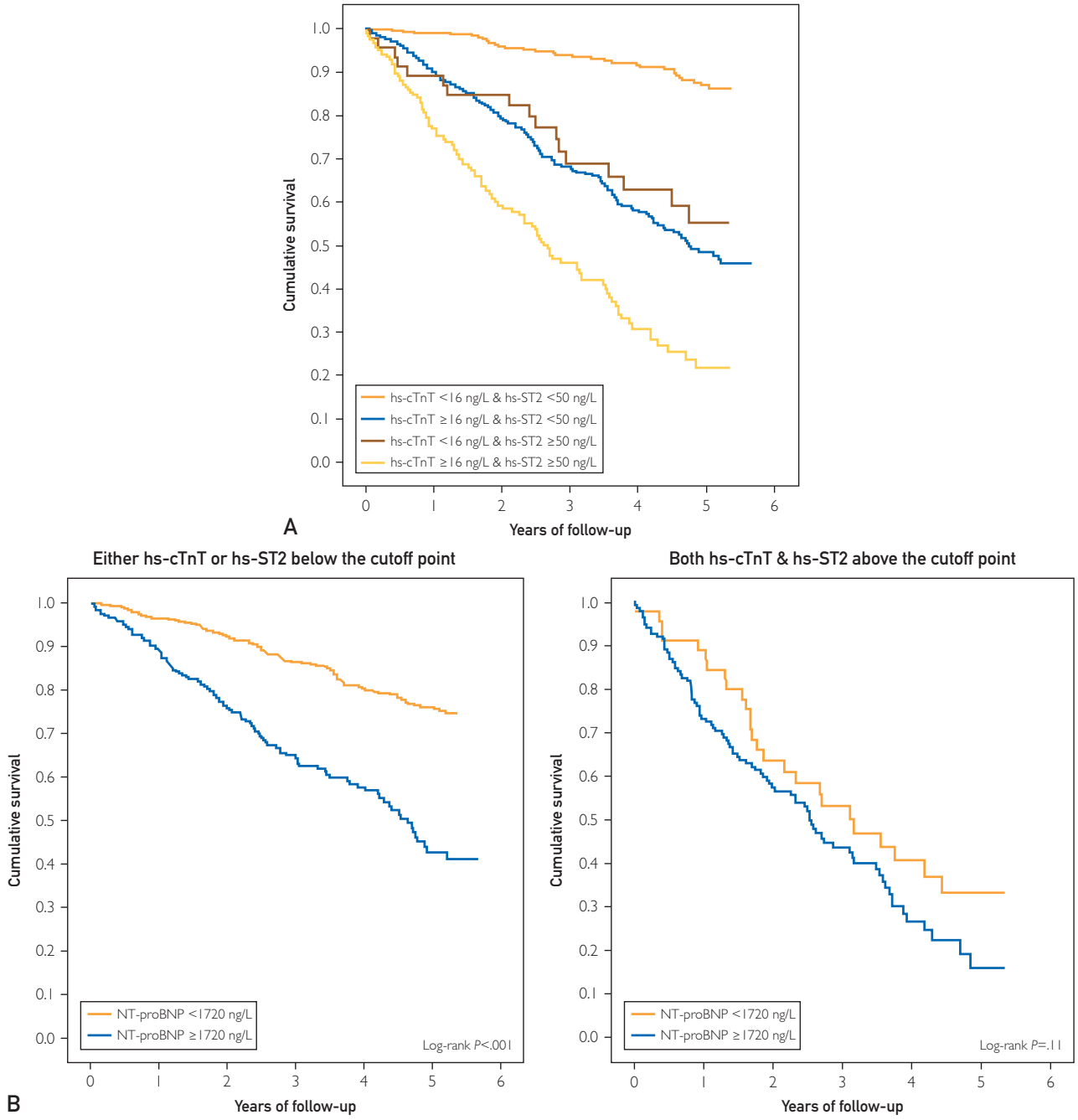

FIGURE 1. Kaplan-Meier survival curves according to biomarkers. A, Survival according to high-sensitivity cardiac troponin T (hs-cTnT) and high-sensitivity soluble ST2 (hs-ST2) levels (above or below the cutoff points). B, Survival according to N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels (above or below the cutoff point) in patients with either hs-cTnT or hs-ST2 levels below the cutoff points (left) and in patients with hs-cTnT and hs-ST2 levels above the cutoff points (right).

TABLE 3. Performance of the Models at 4 Years[a,b]

Variable	Model 1	Model 2	Model 3	Model 4	Model 5
Discrimination					
AUC	0.762	0.780	0.784	0.789	0.790
	(0.736 to 0.789)	(0.755 to 0.805)	(0.759 to 0.808)	(0.766 to 0.813)	(0.766 to 0.813)
	Reference	*P*=.004	*P*<.001	*P*<.001	*P*<.001
Calibration					
H-L	χ^2=8.6	χ^2=12.1	χ^2=12.1	χ^2=7.8	χ^2=12.7
	P=.38	*P*=.15	*P*=.15	*P*=.45	*P*=.12
Brier score	0.161	0.150	0.148	0.144	0.143
AIC	3591	3553	3554	3540	3538
BIC	3643	3619	3620	3611	3614
Likelihood ratio	Reference	*P*<.001	*P*<.001	*P*<.001	*P*<.001
Reclassification					
IDI		3.1 (1.7 to 4.5)	2.7 (1.3 to 4.9)	4.1 (2.5 to 5.6)	4.3 (2.7 to 5.9)
	Reference	*P*<.001	*P*<.001	*P*<.001	*P*<.001
NRI—all		4.2 (−3.0 to 11.3)	9.6 (2.5 to 16.8)	13.9 (6.2 to 21.6)	10.7 (2.6 to 18.7)
	Reference	*P*=.25	*P*=.008	*P*<.001	*P*=.009
NRI—deceased		3.6 (−1.8 to 9.18)	4.1 (−1.6 to 9.9)	7.8 (2.1 to 13.5)	5.4 (−0.8 to 11.6)
	Reference	*P*=.19	*P*=.15	*P*=.007	*P*=.09
NRI—alive		0.5 (−3.6 to 4.7)	5.5 (1.9 to 9.1)	6.1 (1.9 to 10.3)	5.3 (1.1 to 9.5)
	Reference	*P*=.8	*P*=.003	*P*=.005	*P*=.01

[a]AIC = Akaike information criterion; AUC = area under the receiver operating characteristic curve; BIC = Bayesian information criterion; H-L = Hosmer-Lemeshow test; hs-cTnT = high-sensitivity circulating troponin T; hs-ST2 = high-sensitivity soluble ST2; IDI = integrated discrimination improvement; NT-proBNP = N-terminal pro-B-type brain natriuretic peptide; NRI = net reclassification improvement.

[b]Model 1 = age, female sex, ischemic etiology of heart failure, left ventricular ejection fraction, New York Heart Association functional class, diabetes mellitus, estimated glomerular filtration rate, angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker treatment, β-blocker treatment, sodium, hemoglobin; model 2 = model 1 + NT-proBNP + hs-cTnT; model 3 = model 1 + NT-proBNP + hs-ST2; model 4 = model 1 + hs-cTNT + hs-ST2; model 5 = model 1 + NT-proBNP + hs-cTNT + hs-ST2.

[c]All *P* values vs model 1.

any combination of biomarkers compared with the model with established mortality risk factors (*P*<.001), yet the benefit was highest for the combination hs-cTnT + hs-ST2 and with all 3 biomarkers (4.1 [95% CI, 2.5-5.6] and 4.3 [95% CI, 2.7-5.9], respectively; Table 3). Nevertheless, the separate addition of NT-proBNP into a model that already contained hs-cTnT + hs-ST2 did not significantly improve IDI reclassification (model 4 vs model 5; *P*=.34; Table 4).

The NRI (reclassification according to predefined risk categories) significantly improved after the inclusion of hs-ST2 (with either NT-proBNP or hs-cTnT) and with all 3 biomarkers but was not significantly better with NT-proBNP + hs-cTnT (Table 3). The best NRI values were obtained after the addition of hs-cTnT + hs-ST2 (13.9%; 95% CI, 6.2%-21.6%; model 4 in Table 4). Net reclassification indices even worsened after NT-proBNP addition into the full model (model 4 vs model 5 in Table 4).

Crude Mortality

Mortality during follow-up linearly increased from patients without any biomarker elevation (10%) to patients with 3 raised biomarkers above the cutoff points (63.7%) (Figure 2). Mortality for patients with high hs-cTnT + hs-ST2 (above the cutoff points) was 62.2%; however, this combination permitted the identification of 183 patients whereas only 138 patients had all 3 biomarkers above their cutoff points. Twenty-six additional deaths were detected with combining only hs-cTnT + hs-ST2 (114 vs 88). Moreover, if only cardiovascular death was analyzed, cardiovascular mortality for patients with high hs-cTnT + hs-ST2 levels was 34.4%, and this combination permitted the identification of 62 cardiovascular deaths; with all 3 biomarkers above their cutoff points, only 52 cardiovascular deaths were detected.

DISCUSSION

This study provides a comprehensive analysis of the prognostic value of the combination of

TABLE 4. Direct Comparison of Performance at 4 Years of Models Containing Biomarkers[a,b]

Variable	Model 2 vs model 4		Model 3 vs model 4		Model 4 vs model 5	
Discrimination						
AUC	0.780 (0.755 to 0.805)	0.789 (0.766 to 0.813)	0.784 (0.759 to 0.808)	0.789 (0.766 to 0.813)	0.789 (0.766 to 0.813)	0.790 (0.766 to 0.813)
	P=.003		*P*=.23		*P*=.71	
Calibration						
H-L	χ^2=12.1 *P*=.15	χ^2=7.8 *P*=.45	χ^2=12.1 *P*=.15	χ^2=7.8 *P*=.45	χ^2=7.8 *P*=.45	χ^2=12.7 *P*=.12
Brier score	0.150	0.144	0.148	0.144	0.144	0.143
AIC	3553	3540	3554	3540	3540	3538
BIC	3619	3611	3620	3611	3611	3614
Likelihood ratio	NA	NA	NA	NA	Reference	*P*=.04
Reclassification						
IDI		0.9 (−2 to 0.1)		1.4 (0.3 to 2.5)		0.2 (−0.2 to 0.7)
	Reference	*P*=.08	Reference	*P*=.009	Reference	*P*=.34
NRI—all		10.4 (5.1 to 15.7)		5.9 (−0.4 to 12.1)		−2.3 (−5.7 to 1.1)
	Reference	*P*<.001	Reference	*P*=.07	Reference	*P*=.18
NRI—deceased		4.7 (0.8 to 8.7)		4.6 (−0.3 to 9.5)		−2.2 (−4.7 to 0.4)
	Reference	*P*=.02	Reference	*P*=.06	Reference	*P*=.09
NRI—alive		5.7 (2.6 to 8.8)		1.3 (−2.3 to 4.8)		−0.1 (−2.0 to 1.8)
	Reference	*P*<.001	Reference	*P*=.48	Reference	*P*=.90

[a]AIC = Akaike information criterion; AUC = area under the receiver operating characteristic curve; BIC = Bayesian information criterion; hs-cTnT = high-sensitivity circulating troponin T; hs-ST2 = high-sensitivity soluble ST2; H-L = Hosmer-Lemeshow test; IDI = integrated discrimination improvement; NA = not applicable; NT-proBNP = N-terminal pro-B-type brain natriuretic peptide; NRI = net reclassification improvement.

[b]Model 2 = model 1 + NT-proBNP + hs-cTnT; model 3 = model 1 + NT-proBNP + hs-ST2; model 4 = model 1 + hs-cTNT + hs-ST2; model 5 = model 1 + NT-proBNP + hs-cTNT + hs-ST2.

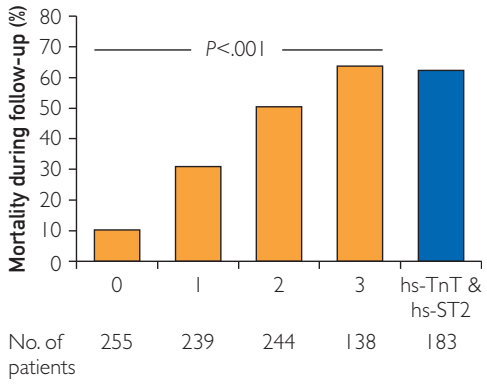

FIGURE 2. Crude mortality rates during follow-up. The presence of any biomarker above the cutoff point (from 0 to 3) is shown on the x-axis. The blue bar illustrates the high-sensitivity cardiac troponin T (hs-TnT) + high-sensitivity soluble ST2 (hs-ST2) combination above the cutoff points.

NT-proBNP (a marker of myocardial stretch), hs-cTnT (a marker of myocardial damage), and hs-ST2 (a marker of myocardial fibrosis and remodeling) in a real-life cohort of patients with chronic HF. All 3 biomarkers were incorporated on top of a model with 11 well-established risk factors (age, sex, ischemic etiology, LVEF, NYHA functional class, diabetes mellitus, eGFR, sodium level, hemoglobin level, β-blocker, and ACEI and ARB treatments). The AUC for this model was 0.76, which compares favorably with other proposed models in HF (ie, the community validation of the Seattle Heart Failure Model had an overall AUC of 0.73).[24]

The addition of 2 or more biomarkers from different pathophysiologic pathways into a multimarker analysis to obtain additive prognostic information in HF seems to be a rational and reliable strategy for identifying patients who need to be followed up more closely.[5,6,16-18] Most multimarker strategies have involved adding the combination of other biomarkers to the well-established BNP or NT-proBNP, given the usefulness of these peptides in a wide range of HF situations.[6] Second-generation biomarkers include hs-cTnT and hs-ST2. Cardiac troponin

is a marker of myocyte injury, and the hs-cTnT assay has recently become available for detecting extremely low troponin concentrations and improving precision at the lower limit of detection.[21] ST2 is a biomarker for myocardial fibrosis and remodeling; under the induction of separate promoters, the ST2 gene expresses two unique proteins: soluble ST2, which is the circulating form of ST2 (as assessed in this study), and ST2 ligand, which is the transmembrane form of the protein that signals through a complex involving interleukin 33.[25]

The additional prognostic information gained by any marker over a clinical risk model plus other biomarkers needs to be determined using adequate statistical tools.[26] A major problem in selecting a biomarker profile is the proportional increase in economic burden,[6] so the addition of any biomarker to a profile should be justified by adequate discrimination, calibration, reclassification, and likelihood analyses. According to the present results, the combination of an increasing number of biomarkers did not necessarily improve risk stratification in HF.

The usefulness of combining natriuretic peptides with either hs-ST2 or hs-cTnT has been reported previously,[18,27,28] but not the combination of the 3 in the setting of chronic HF. In this study, we found that hs-cTnT + hs-ST2 performed as well as or better than the combination of all 3 added biomarkers (NT-proBNP + hs-cTnT + hs-ST2). The different analysis yielded 3 relevant findings. First, NT-proBNP added to hs-cTnT + hs-ST2 did not improve prognostic accuracy or reclassification indices. Second, NT-proBNP increased prognostic discrimination only in patients with either hs-cTnT or hs-ST2 levels below the cutoff point. Third, the combination of hs-cTnT + hs-ST2 identified more decedents during follow-up than did the combination of the 3 biomarkers. The latter was observed even when only cardiovascular deaths were taken into account. Together, these main findings suggest that the pathways identified by hs-ST2 and hs-cTnT profoundly affect mortality in the context of chronic HF, whereas the information provided in their presence by natriuretic peptides might be redundant. However, as shown in this study and depicted in Figure 1, B the separate addition of NT-proBNP provided prognostic discrimination in patients with either hs-cTnT or hs-ST2 levels below the cutoff point.

Although modification of some mortality risk factors may decrease the risk of HF hospitalizations and death, evidence is lacking that reducing the levels of hs-cTnT and hs-ST2 will reduce risk. Therefore, these data should not be construed as implying a direct benefit from reducing biomarker levels. A better risk assessment is clinically of great value as it more accurately identifies patients with HF at increased risk of death who could then be targeted for more intensive monitoring and treatment.

In this study, we analyzed only 1 blood sample and cannot comment on the prognostic value of serial determinations. The population was a general HF population treated at a specific and multidisciplinary HF unit in a tertiary care hospital; most patients were referred from the cardiology department and, thus, were relatively young men with HF of ischemic etiology and reduced LVEF. As such, these results cannot necessarily be extrapolated to a global HF population.

CONCLUSION

A new generation of biomarkers (hs-cTnT and hs-ST2) for different pathophysiologic processes from those of natriuretic peptides perform as well as or better for risk stratification in chronic HF. If these results are validated, the incorporation of these biomarkers into clinical practice for the prediction of death could be accomplished quickly. Further studies should confirm whether natriuretic peptides might be nonmandatory for HF risk stratification.

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Abbreviations and Acronyms: **ACEI** = angiotensin-converting enzyme inhibitor; **AIC** = Akaike information criterion; **ARB** = angiotensin II receptor blocker; **AUC** = area under the receiver operating characteristic curve; **BIC** = Bayesian information criterion; **eGFR** = estimated glomerular filtration rate; **HF** = heart failure; **HR** = hazard ratio; **hs-cTnT** = high-sensitivity cardiac troponin T; **hs-ST2** = high-sensitivity soluble ST2; **IDI** = integrated discrimination improvement; **LVEF** = left ventricular ejection fraction; **NRI** = net reclassification improvement; **NT-proBNP** = N-terminal pro-B-type natriuretic peptide; **NYHA** = New York Heart Association; **P_{25-75}** = 25th-75th percentiles

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